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Out of the jaws of death: PRMT5 steers p53

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Abstract

The tumour suppressor p53 triggers either cell-cycle arrest or apoptosis. Now, arginine methylation joins a panoply of other post-translational modifications that regulate p53. PRMT5 mediates p53 methylation, which disposes the cell to arrest rather than death.

Tumour suppressors are important surveillance proteins, guarding against unchecked cellular growth that is potentially detrimental to the organism. p53 is a key tumour suppressor in human cells, as indicated by mutation of the p53 gene in most types of cancer¹. Because of the crucial nature of p53 in human health, enormous effort has been expended to fully understand how it works and how it is regulated. Most of the physiological functions of p53 are attributable to its role as a transcriptional activator, responding to agents that damage DNA². p53 target genes have two alternative downstream effects: either they pause the cell cycle, allowing the DNA to be repaired, or, if repair is not possible, they activate processes leading to apoptosis, that is, programmed cell death. How p53 ‘chooses’ between these distinct outcomes is a central question in the field of tumour biology³.

A major clue to the gene regulatory mechanism of p53 was revealed through mapping locations of mutations in patients. These occur mainly in the DNA binding domain of p53, which occupies nearly one-third of the central portion of the protein. It is clear that loss of DNA binding obliterates function, and thus it has been relatively straightforward to show that DNA binding is crucial for the role of p53 as a tumour suppressor⁴. However, there are numerous additional discrete functional domains: two amino-terminal transcriptional activation domains and several domains in the carboxy terminus, including nuclear import/export signals, a tetramerization domain and a poorly defined regulatory domain².

Interestingly, p53 is also replete with post-translational modifications (PTMs)⁵, that is, chemical groups covalently added to proteins to subtly or sometimes dramatically alter their function. Phosphorylation was one of the first PTMs to be clearly defined on p53, mainly because key signalling pathways responsive to DNA damage, such as those involving the kinases ATM and ATR, lead to p53 phosphorylation⁶. It is noteworthy that most of the phosphorylation sites reside in the N- and C-terminal domains rather than in the DNA damage domain, and modulate the degree of p53 function, rather than being a binary on/off switch. In the last decade it has become additionally clear that p53 is modified not only by phosphorylation, but that it is extensively modified by lysine acetylation and methylation, among other modifications^{5,7,8}. Indeed, besides histone proteins (where specific acetylation/methylation sites were first matched with cognate enzymes), p53 is the most common protein substrate known for these PTMs.

However, despite the plethora of PTMs, p53 has never been identified as a substrate for arginine methylation. This is surprising because arginine methylation sites, and relevant enzymes, have been known for a long time for proteins that interact with RNA, including splicing and ribosomal proteins⁹. In addition, a number of transcription factors and histones

are arginine methylation substrates. On page 1431, Janssen *et al.*¹⁰ identify PRMT5 as a p53 arginine methylase. The authors purify a p53 cofactor called Strap and discover that PRMT5 is physically associated with Strap. (Fig. 1a). They had found previously that Strap cooperates with another cofactor called EMY, facilitating the interaction between p53 and the well-known coactivator and histone acetyltransferases CBP and p300 to activate transcription. EMY and Strap seem to be direct cofactors that stabilize p53 protein levels and augment p53 activity. The authors then set out to determine whether PRMT5 directly methylates p53. They showed that PRMT5 methylates p53 *in vitro*, and mapped the sites of methylation using mass spectrometry of cellular p53. They developed an antibody that specifically detects p53 methylated on these sites (Arg 333, 335 and 337) and confirmed that p53 is methylated *in vivo*; methylation requires PRMT5 and is increased in response to etoposide, a DNA damaging agent. As with most of the p53 modifications sites, the methylation sites are outside the DNA binding domain, mapping to the C-terminal region that encompasses the nuclear localization and tetramerization domains. The authors found that PRMT5 promotes p53 oligomerization and targeting to the nucleus, which is dependent on the methylation sites. Further, PRMT5 and the methylation sites stimulate p53-dependent G1 arrest in response to DNA damage, but they do not affect the p53-dependent apoptotic response. The cell-cycle effect depends on activation of *p21* gene expression, a p53-dependent gene required for G1 arrest, whereas little effect was detected on genes mediating apoptosis. Thus, it seems that PRMT5-mediated arginine methylation of p53 may help to discriminate between the cell-cycle response and the apoptotic response. These results raise the question of whether p53 is methylated only when bound to genes that promote cell-cycle arrest to explain the selectivity of the p53 response (Fig. 1b).

A persistent, controversial and sometimes vexing question is just how crucial are the p53 PTMs for its function¹¹. Single or even multiple substitutions have a modest effect on function, compared with certain substitution(s) in the DNA binding domain, as mentioned above. Indeed, Janssen *et al.* have found only rare substitutions of these arginine sites in human disease¹⁰. One obvious possibility is that the PTMs are redundant in that they may work together in groups, for example to recruit effector proteins, such as histone modifiers, and thus single substitutions do not abrogate function. This may be the case for PRMT5 methylation, as Janssen *et al.* showed that there are three closely clustered arginine methylation sites (Fig. 1a); simultaneous substitution of all three lowers activity of p53 in cell-cycle arrest (although single substitutions were not tested)¹⁰. Similarly, it was shown recently that substitution of all known lysine acetylation sites — and most of these are alternatively methylated as well — markedly lowers p53-mediated gene activation¹². Previously, it was noted that loss of a smaller number of lysines does not cripple p53 function¹¹. Perhaps the collective activity of PTMs classes may be the crucial underlying functional feature.

There are numerous issues for future investigations emerging from this study. One question is the precise biochemical effect on p53 protein: is Prmt5-mediated methylation required for p53 nuclear localization or oligomerization? In either case this would presumably increase p53 binding to every gene target, so how would this pathway specifically upregulate promoters of genes involved in cell-cycle arrest, and not gene targets promoting apoptosis? One possibility is that the PRMT5-associated cofactors Strap and JMY (Fig. 1a) might stimulate p53 binding to specific subsets of target promoters involved in cell-cycle arrest.

Second, is there an interplay of p53 arginine methylation by PRMT5 with the myriad other PTMs on p53 (ref. 5)? The sites of arginine methylation are very close to an acetylation site at Lys 320, and they are adjacent to the C-terminal regulatory domain harbouring the p300/CBP-dependent acetylation sites (Lys 370/Lys 382). Indeed, as Strap also interacts with p300/CBP (Fig. 1a), it is probable that the arginine methylation promoted by Prmt5 will

functionally interact with the lysine acetylation by p300/CBP (Fig. 1b). Further, arginine methylation of histones regulates transcription¹³, including p53-mediated transcription¹⁴, raising the question of whether there is crosstalk between p53 methylation and histone methylation through PRMT5. p53 acetylation is required for histone acetylation¹⁵, and in the same way, p53 arginine methylation may regulate histone arginine methylation through PRMT5 (Fig. 1b).

Finally, the results of this study show compellingly that PRMT5-mediated arginine methylation of p53 is important in a cell culture setting. Nonetheless, it will be essential to determine the role of this pathway *in vivo*, for example by preparing mouse *p53* knock-in mutants bearing substitutions at potential PTM sites. It will be fascinating to test the importance of these arginine methylation sites *in vivo*, and their interplay with other modifying enzymes and PTM sites.

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Figure 1. PRMT5 regulation of p53

(a) PRMT5 mediates arginine methylation of p53. PRMT5 associates with the cofactor Strap, which associates with additional cofactors JMY and p300/CBP. These cofactors associate with p53. Sites of methylation on p53 are shown. (b) Coordination of p53 modifications. P300/CBP acetylates p53, which leads to histone acetylation. Similarly, PRMT5 methylation of histones may be triggered by p53 methylation.